[Cell Adhesion & Migration 3:2, 146-147; April/May/June 2009]; ©2009 Landes Bioscience

Journal Club

Shaping the gradient by nonchemotactic chemokine receptors

Elena Monica Borroni and Raffaella Bonecchi*

Department of Translational Medicine; University of Milan; Istituto Clinico Humanitas IRCCS; Rozzano, Italy

Key words: Chemokine, chemokine receptor, leukocyte recruitment, chemotaxis, transcytosis

Chemokines are a class of inflammatory mediators which main function is to direct leukocyte migration through the binding to G protein–coupled receptors (GPCRs). In addition to these functional, signal-transducing chemokine receptors other types of receptors belonging to the chemokine GPCR family were identified. They are called atypical or decoy chemokine receptors because they bind and degrade chemokines but do not transduce signals or activate cell migration. Here there is the summary of two recent papers that identified other nonchemotactic chemokine receptors: the Duffy antigen receptor for chemokines (DARC) that mediates trancytosis of chemokines from tissue to vascular lumen promoting chemokine-mediated leukocyte transmigration and chemokine (CC motif) receptor-like 2 (CCRL2) that neither internalizes its ligands nor transduces signals but presents bound ligands to functional signaling receptors improving their activity. Collectively these nonchemotactic chemokine receptors do not directly induce cell migration, but appear nonetheless to play a nonredundant role in leukocyte recruitment by shaping the chemoattractant gradient, either by removing, transporting or concentrating their cognate ligands.

Introduction

Chemokines are key regulators of leukocyte migration from the blood into sites of inflammation activating integrin adhesiveness and inducing chemotaxis through the endothelium. To activate these functions chemokines produced in the inflamed tissue must be transcellularly transported across the endothelial barrier and tethered to the endothelium. Moreover chemokines need to be bound to structures within the tissue to regulate the formation of chemotactic gradient. Though these biological processes have been clearly demonstrated, the underlying molecular basis is poorly understood.[1] Two recent publications resumed here indicate that nonchemotactic chemokine receptors may account for these biological functions.

DARC: chemokine transcytosis and tethering to the endothelium. Originally described as the erythrocyte receptor for malaria parasites, DARC was later identified as the erythrocyte receptor for CXCL8 and is now recognized as an atypical chemokine receptor able to interact with a large panel of pro-inflammatory CXC (e.g. CXCL1, CXCL3, CXCL5, CXCL6, CXCL8) and CC (e.g. CCL2, CCL5) chemokines, but not with homeostatic chemokines. When expressed on erythrocytes, DARC modulates chemokine bioavailability by acting as a chemokine "sink" and as a long-term blood reservoir of chemokines that prevents their loss into distant organs and tissues.[2] However, DARC is also expressed by endothelial cells of postcapillary venules in kidney and spleen and by high endothelial venules in lymph nodes and tonsils. Pruenster et al.[3] investigate DARC subcellular distribution and function using Madin-Darby canine kidney (MDCK) cells overexpressing DARC (MDCK-DARC cells) grown polarized on Transwell filters. Using confocal microscopy and immunoelectron microscopy authors demonstrate that DARC is expressed both on the basolateral and apical membrane, after chemokine engagement it is rapidly targeted into caveolae and internalized, and progressively accumulates on the apical side of the cells where immobilizes and presents the intact chemokine. Using the same experimental setting authors demonstrate that DARC-expressing monolayers transcytate chemokines from the basolateral to the apical direction and support increased chemokine-induced leukocyte transmigration compared to monolayers transfected with the empty vector (MDCK-mock cells). Similar results are obtained by the authors using immortalized human umbilical vein endothelial cells (HUVEC) DARC transfected. By the use of transgenic mice overexpressing DARC on endothelial cells (mDARCtg), authors demonstrate increased neutrophil extravasation compared to wild type littermates in response to intraperitoneal and intradermal injection of the DARC ligand mouse CXCL1. In order to test DARC function in a disease setting of inflammatory pathology, authors perform a classical contact hypersensitivity (CHS) experiment comparing ear thickness and weight between wild type and mDARCtg mice. They find that mDARCtg mice have increased ear swelling due to enhanced leukocyte infiltration evaluated by histological examination of CHS lesions.

CCRL2: chemokine tethering and presentation by mast cells. Human CCRL2 is located on the chromosome 3 at the end of a chemokine receptor gene cluster containing CCR1, CCR3, CCR2 and CCR5 and its aminoacid sequence is strictly related to these signaling receptors. However, like chemokine decoy receptors, it lacks highly conserved motifs like the so called DRYLAIV motif in the second intracellular loop and an aspartic residue in the second transmembrane domain and for this reason it was postulated to be a silent/decoy receptor.[4] Zabel et al. report in a recent paper[5] that CCRL2 binds with high affinity the chemoattractant molecule

*Correspondence to: Raffaella Bonecchi, PhD; Istituto Clinico Humanitas; Immunology; Via Manzoni 113; Rozzano, Milano I-20089 Italy; Tel.: +39.02.8224.5117; Fax: +39.02.8224.5101; Email: raffaella.bonecchi@humanitas.it

Submitted: 2/10/09; Accepted: 2/24/09

Previously published online as a *Cell Adhesion & Migration* E-publication: www.landesbioscience.com/journals/celladhesion/article/8280

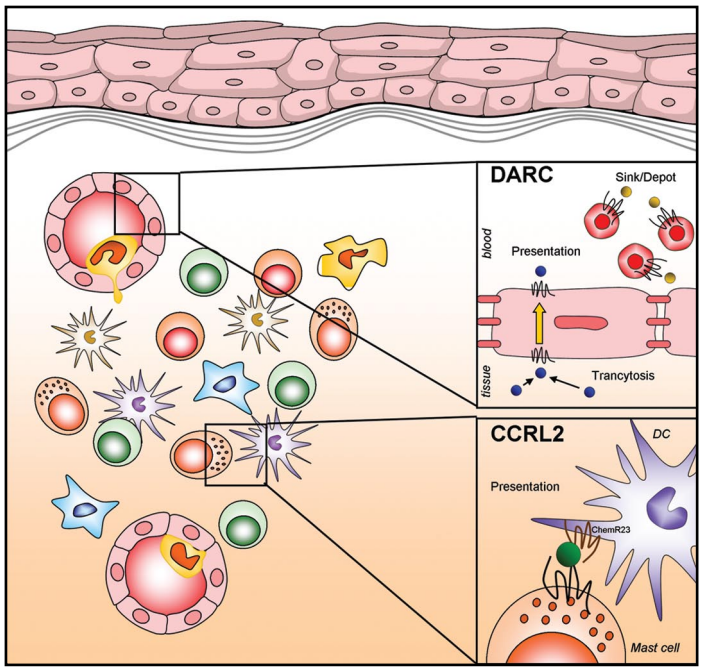

Figure 1: Schematic representation of the nonchemotactic chemokine receptors DARC and CCRL2. DARC might have several mechanisms of action, including acting as a depot for chemokines on erythrocytes and as a transporter of chemokines from the basolateral to apical side of endothelial venules where presents them to leukocytes. Conversely, CCRL2 is selectively expressed on mast cells where is involved in chemerin immobilization and presentation to ChemR23-positive dendritic cells.

chemerin. Surprisingly they find that, differently from the other two other chemerin receptors, ChemR23 and GPR1, after chemerin binding CCRL2 does not activate chemotaxis signal and it is not internalized. Using truncated chemerin forms authors demonstrate that CCRL2 binds the N-terminal domain of chemerin, a portion of the molecule that is not involved in ChemR23 binding and activation. Furthermore they show that CCRL2 expressing cells preloaded with chemerin induced functional response in ChemR23 transfectants, indicating that the chemoattractant is still functional after CCRL2 binding. The in vivo results obtained with CCRL2-/- mice support the hypothesis that CCRL2 might function as a chemerin concentrator. Authors focus their analysis on mast cells that express high levels of CCRL2 in basal conditions. BM-derived cultured mast cells (BMCMCs) from wt or CCRL2-/- mice do not display differences in basic mast cell functions such as degranulation, cytokine secretion and stimulation of T cell proliferation. On the contrary, using a model of atopic allergy, IgE-dependent passive cutaneous anaphylaxis (PCA) reaction, CCRL2-/- mice have reduced inflammation when sensitized with a low dose of Dinitrophenyl (DNP)-specific IgE. By the use of a mast cell–deficient mice engrafted with wt or D6-/- BMCMCs authors demonstrate that the defect is due to the lack of CCRL2 on mast cells.

Nonchemotactic chemokine receptors have been initially defined as silent referring to the lack of conventional functional and biochemical responses.[4] However it is emerging that some of them transduce G-protein-independent signaling pathways in association with internalization that regulate their surface expression in order to optimize their chemokine scavenging ability.[6] The pubblications resumed here do not explain the biochemical properties of DARC and CCRL2, in particular how chemokines are sorted to the transcytotic pathways or how chemokines bound to these receptors are retained and concentrated on cell surface. Moreover for DARC-mediated chemokine transcytosis strong genetic evidence is still missing. Nevertheless these publications clearly indicate that CCRL2 and DARC may be considered members of a new class of atypical chemokine receptors that, similarly to glycosaminoglycans (GAGs)[7] present chemokines to conventional signal-trasducing chemokine receptors optimizing their activity. It is worth to note that despite functional heterogeneity all nonchemotactic chemokine receptors removing, transcytating or concentrating chemokines play a nonredundant role in leukocyte recruitment actively participating in the formation of chemotactic gradients.

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